### Date: December X, 2017

Wieslaw Bogdanowicz

Editor-in-Chief, Acta Chiropterologica

## Subject: Re-submission of manuscript AC-00201-2017-01

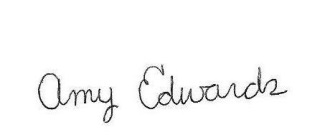
Dear Dr. Bogdanowicz,

We are pleased to re-submit our manuscript entitled “**Mercury concentrations in bat guano from caves and bat houses in Florida and Georgia, USA”** authored by Amy Edwards, Jenise Swall and Charles Jagoe for possible publication in Acta Chiropterologica.

We responded to each Reviewer’s comments below (bolded text), with corresponding changes below (plain text) and in the revised manuscript.

Please contact me at [*aeedwards@hanovercounty.gov*](mailto:aeedwards@hanovercounty.gov)if you have any questions or need more information. Thank you very much for this opportunity to re-submit our manuscript.

Sincerely,



Amy E. Edwards, Ph.D

**Review 1:**

**General Comments:**

**The manuscript needs a formal editing for diction, sentence structure, and typos:** The authors edited the paper for diction, sentence structure and typos.

**The author names and affiliations do not appear on the manuscript:** I could’ve sworn that I was asked to remove this information during manuscript submittal, but I would have to start the submittal process on the website for a new manuscript to check on this. I know for sure the acknowledgments needed to be removed, as that was stated in the instruction for authors. I suppose I’ll just put the names and affiliations in the re-submitted manuscript and make a note of it here. The author names and affiliations are in the re-submitted manuscript.

**Key Words needs to be expanded:** The authors added key words.

**The study has potential but currently is not presented in a way that is fully appropriate or complete and has issues with data collection and analysis:** This is vague. Not sure how to respond. The authors have edited the text to clarify hypothesis and study methods.

**Abstract: Should be expanded to better state the methods and conclusion of the study. Does not provide any statistical analysis:** The abstract was expanded to better state methods and conclusion of the study, including the method of statistical analysis.

**Introduction: The introduction covers most of the relevant literature. However, does not provide any hypotheses and prediction:** I put a hypothesis in the introduction, and a prediction that the assumed modern guano in Florida would have similar concentrations to the guano in the Hagan 2014 Kentucky study (the bats are assumed similar insectivorous species). Hypotheses and prediction were added to the Introduction.

**Methods and Materials: Widely disparate sampling make statistical comparison difficult. Why were only two "house" colonies sampled as opposed to 10 caves:** The state of Florida only has two bat house colonies known to the authors that are available for sampling, while there are hundreds of caves. Cave sampling was limited by permission to access and take samples in the cave, as well as history of known bat habitation in the cave.

**Authors need to report all equipment used, inherent error, manufacturer, and city of origin:** I have never read a paper that had all this information… how do I handle this question? I don’t remember the brand of corer, so I would need to contact Dr. Hsieh to find out this information.Do I need to do this for the DMA 80 as well?

**The authors need to state what type of plastic bags the samples were stored in as some plastics can leach mercury if colored:** The reclosable, Ziplock plastic bags used for storing samples were clear and not colored. This information was added to the Materials and Methods section.

**There is no mention in the methods section of statistical test to be applied:** The methods section focuses on the data collection and measurement process. The results section focuses on the statistical results that we obtain based on the measurements that were made. The idea is that, at the time that data is collected, the researcher may envision using certain statistical techniques, but later find that those techniques are not appropriate. This answer is also intended to address the next comment (below).

**Results: The results section contains information that should be presented in the methods section:** I think the reviewer is talking about the software used (R), and maybe the name of the specific tests, like Welch’s ANOVA, so I put this information in the methods section. Information from the Results section was moved and/or added to the Results section.

**The authors mention a "correlation" analysis but do not provide the results ("did not find evidence that measurements from the same core consistently showed more correlation than any other group of samples from the same cave or bat house."):** Thank you for pointing out that this was not clear. We have added more explanation to clarify this.

**The authors combine all samples to produce a mean mercury estimate, but pooling data from different sites requires that the means among samples of the sites are not significant. This was not done:**

As the reviewer points out, some of the means were significantly different. To address this concern, we have utilized a generalized least squares model, which allows for different means and variances for the individual caves/bat houses. We can then use linear contrasts and linear combinations of the estimates to ascertain the mean across bat houses, the mean across caves, and the overall mean.

**The authors state that there was significant differences between house and cave mean mercury levels, but do not state what test was used and what assumption of the test were met. It is unlikely that the assumptions of any parametric test would be met by such a data set:**

The generalized least squares model (described in the response to the previous comment), which allows for separate means and variances for each cave/bat house, is a reasonable choice for this dataset. However, this model does still assume normality, though such models are usually robust to violations of this assumption as long as there are no serious violations. However, since some of our sample sizes are small, we decided to compare this model against a Bayesian hierarchical model (also with separate means and variances), that used a t-distribution with 4 d.f. for the data likelihood component. This use of the t-distribution, which cannot be done in generalized least squares, allows for more frequent outliers and heavier tails in the distribution. The results of these models were in close agreement, which makes lends additional confidence to the results of our generalized least squares model. We decided not to include the Bayesian strategy here, as each Bayesian model is customized for an application and must be programmed by the user. A major strength of the generalized least squares approach is the fact that many software packages have a built-in routines for fitting such models.

**Comparisons among caves may be warranted, however, there are differences in the number of samples taken among caves which makes these data circumspect.** Collecting an equal number of samples among the caves was a theoretical part of the study design before sampling started. However, due to the heterogeneous nature of the quantity and depth of guano available in the caves sampled, the number of samples were different. This was added to the Methods section.

**In addition, the authors do not state how their sampling was randomized at each site. All sites should have had randomized sampling efforts and the number of samples taken should have been standardized depending on the amount of guano that was present. The means and standard deviations from the samples gathered at each site should be calculated and a test for the variance across sites would tell the authors if they can combine (pool) data from all sites:** Core sampling was chosen randomly based on the depth of the guano piles, and few guano piles in the caves or bat houses were deep enough to use the corer. Collection sites were approximated on cave survey maps, and the authors can include these maps as figures if requested. Calculating the amount of guano present in the caves would have been difficult if not impossible, as the breadth and depth of guano was different for every cave and would have required extensive time and damage to the cave environments. Bats were also present in several of the caves during guano collection, and the authors wanted to keep disturbance to the bats minimal.

**The authors state that they limited their comparisons to six caves with "sufficient samples," but do not state how this was determined:** Over a hundred samples of guano and cave sediment were collected from more than six caves for the total study, but only samples from actual guano piles were presented in this manuscript. Only six caves had enough guano samples (> 2 observations) to allow estimation of a mean and variance for that cave..

**Discussion: There is a fundamental difference between maternity and hibernating colonies in terms of sex, age, and ecology of resident bats and although the authors mention in the introduction that they sampled from both types of colonies, they do not address these differences. I think adding this would help the manuscript and also help delineate proper statistical testing. Colony sizes of bats also will affect the outcomes of the study and these data are not given or discussed:**  Detailed data on the bat population in the caves or bat houses was not collected during the study and is not available to the authors. The goal of the study was not to be a comprehensive study on mercury in bat guano correlated with bat species, sex, or age, but to make a preliminary analysis of mercury concentrations in bat guano in Florida and Georgia to compare the results with modern, historic and ancient concentrations in bat guano analyzed in a nearby state. Results from the Hagan (2014) study show greater mercury concentrations in modern bat guano, and the modern bat guano in Florida is showing comparable concentrations. The main bat species utilizing caves and bat houses is different, so these two environments were used for a general species comparison.

**In addition, guano piles are not static, dead entities. There are many arthropods that live in guano and burrow and mix up the layers deposited by bats over many years. How does this affect the method of coring as a stratified measure of time?:** The core subsamples were not age-dated, and all guano was considered modern (<100 years) for the purposes of the study, since the guano in Florida caves is subject to flooding and impacted by the fauna that interact with the guano piles.

**References: Adequate:** I want to say something sarcastic, but I’ll bite my tongue and leave this blank when re-submitting ☺

**Review 2**:

**The experimental uncertainty (standard deviation) should be one significant figure (almost always). When the leading digit has a value of 1, it can be extended to a second digit.**

**Thus, 0.1104 +/- .1036 should be reported as 0.11 +/- 0.10 and**

**0.5306 +/- .0403 should be 0.53 +/- .04:**

**While the instrumental method used does provide 4 significant figures and the statistical package does provide the reported standard deviation, the significance (in terms of significant figures) of the reported value should be adjusted appropriately for all results in the paper. The standard deviation reported probably reflects natural variations in mercury concentrations in the samples:**

As suggested by the reviewer, we have changed the number of figures reported.

**More details regarding the extent of the QA/QC protocols would serve to enhance the methodology section:** I’m not sure what this reviewer wants… a more detailed lab QA/QC? The lab part of the QA/QC already passed muster in a previous publication, and I don’t know how to get more detailed with the sampling part without maybe naming the brand of core sample used? Thoughts?